IS 11479-2 (2001): Antibacterial Toilet Soap, Part 2: Liquid [CHD 25: Soaps and other Surface Active Agents]

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भारतीय मानक

जीवाणुरोधी प्रसाधन साबुन — विशिष्टि

भाग 2 द्रव

Indian Standard

ANTIBACTERIAL TOILET SOAP — SPECIFICATION

PART 2 LIQUID

ICS 71.100.40

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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Price Group 6

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soaps and Other Surface Active Agents Sectional Committee had been approved by the Chemical Division Council.

Human skin provides a favourable environment for the existence and multiplication of a variety of microbes. The conventional toilet soap washes away the germs but does not kill them. The function of an antibacterial or antiseptic toilet soap is not only to clean the skin, but also to reduce drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of perspiration by bacteria.

The antibacterial toilet soap is specially effective against *staphylococcus* and similar bacteria which have the habit of residing in the under layers of skin. The antibacterials are substantive to the skin and this tackles the microbes between two washes. Antibacterial toilet soaps shall be used regularly to be effective.

In the present revision hexachloroprene has not been permitted to use as antibacterial agent. Trichlorocarcanilide (TCC) on heating decomposes to chloroanilines which can be harmful to skin and hence the limit and method for determination of chloroaniline is incorporated. It is decided to publish this standard as Part 1(solid cake) and antibacterial liquid toilet soap will form Part 2.

A scheme for labelling environment friendly products known as ECO Mark has been introduced at the instance of the Ministry of Environment and Forests (MEF), Government of India. The ECO Mark would be administered by the Bureau of Indian Standards (BIS) under the *BIS Act*, 1986 as per the Resolutions No. 71 dated 21 February 1991 and No. 425 dated 28 October 1992 published in the Gazette of the Government of India. For a product to be eligible for marking with ECO logo, it shall also carry the ISI Mark of BIS besides meeting additional environment friendly requirements. The requirements to be satisfied for a product to qualify for the BIS Standard Mark for ECO friendliness, has been included in this revision. These requirements will be optional; manufacturing units will be free to opt for the ISI mark alone also.

The requirements of the conventional grade of toilet soaps are given in IS 2888 : 1983 'Specification for toilet soap (*second revision*)'.

There is no ISO specification on this subject. This standard is formulated based on indigenous technology and data available.

The composition of the Committee responsible for formulation of this standard is given in Annex E.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

ANTIBACTERIAL TOILET SOAP — SPECIFICATION

PART 2 LIQUID

1 SCOPE

This standard prescribes requirements and method of sampling and test for antibacterial liquid toilet soap. It does not cover shampoo and products intended for specific purpose such as those for industrial and surgical uses.

2 REFERENCES

The Indian Standards listed below contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
286 : 1978	Methods of sampling and test for soaps (*second revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
4199:1990	Liquid toilet soap — Specification (*second revision*)
4707 (Part 1) : 1986	Classification of cosmetic raw materials and adjuncts : Part 1 Dyes, colours and pigments (*first revision*)
4955 : 1993	Household laundry detergent powders — Specification (*third revision*)
7597 : 1974	Glossary of terms relating to surface active agents
11479 (Part 1) : 1998	Antibacterial toilet soap — Specification: Part 1 Solid cake
13424 : 1992	Safety evaluation of bathing bars and toilet soap — Method of test

3 TERMINOLOGY

For the purpose of this standard, the definitions given in 2 of IS 7597 shall apply.

4 REQUIREMENTS

4.1 Description

The material shall consist essentially of an aqueous solution of potassium soaps, sodium soaps, or both, made from oils, fatty acids or their mixture. It shall be a homogeneous clear, translucent or opaque liquid with good lathering and cleaning properties. It may contain permissible synthetic detergents.

4.2 The material shall quickly form a satisfactory lather while in use.

4.3 The material shall remain as homogeneous stable product and shall show no sign of separation or sedimentation when kept at 5°C for 24 h.

4.4 The material may be suitably perfumed as agreed to between the purchaser and the supplier and if unperfumed, it shall have no disagreeable odour.

4.5 The material may contain permitted colour as given in Table 4 of IS 4707 (Part 1), preservatives, medicaments and such additional substances as are declared on the label. The materials shall be non-injurious to skin when tested by the methods prescribed in IS 13424.

4.5.1 The antibacterial soap shall contain permitted antibacterial agents (*see* **4.5.2**). The soap shall pass anti-bacterial activity test (sensitivity test) when determined by the method given in Annex A.The soap shall meet the requirements for zone of inhibition and contact kill as given in Table 1.

4.5.2 Triclosan (TCN) and Trichlorocarbanilide (TCC) shall not exceed 1 percent by mass either singly or in combination when tested either by the methods prescribed in Annex B.

NOTE — TCC is not heat stable and decomposes into chloroanilines on prolonged heating above 60°C. If TCC is used in soap, the manufacturer should take care that such soap is not subjected to temperature above 60°C during the entire manufacturing process or during storage.

4.6 The material shall show no sign of any deterioration on storage in original sealed containers under normal conditions for a period of six months.

4.7 The material shall also comply with the requirements specified in Table 1 when tested by the methods specified in col 4, 5 and 6 of Table 1.

4.8 The anti-bacterial toilet soap shall pass the test for dermatological safety when evaluated as per the method prescribed in IS 13424.

Table 1 Requirements for Anti-Bacterial Liquid Toilet Soap

(*Clauses* 4.5.1 *and* 4.7)

Sl No.	Characteristic	Requirement	Method of Test, Ref to		
			Clause No. in IS 286	IS	Annex of this Standard
(1)	(2)	(3)	(4)	(5)	(6)
1)	Total fatty matter, percent by mass, *Min*	15.0	15	—	—
ii)	Matter insoluble in alcohol, percent by mass, *Max*	5.0	5	—	—
iii)	Free caustic alkali (as K_2O), percent by mass, *Max*	0.03	—	Annex A of IS 4199	—
iv)	Synthetic detergents, percent by mass, *Max*	2	—	Annex B of IS 4955	—
v)	Zone of inhibition	To pass the test	—	—	C
vi)	Contact kill	To pass the test	—	—	D

4.9 Additional Requirements for ECO Mark

4.9.1 *General Requirements*

4.9.1.1 The product shall conform to the requirements for quality, safety and performance prescribed under **4.1** to **4.7**.

4.9.1.2 The manufacturer shall produce to BIS environmental consent clearance from the concerned State Pollution Control Board as per the provisions of *Water (Prevention and Control of Pollution) Act*, 1974 and *Air (Prevention and Control of Pollution) Act*, 1981 along with the authorization, if required, under the *Environment (Protection) Act*, 1986 while applying for ECO Mark.

4.9.2 *Specific Requirements*

4.9.2.1 The antibacterial toilet soap shall neither contain any synthetic detergent when tested as per the method given in Annex B and C of IS 4955 nor any phosphate when tested as per the method prescribed in **20** of IS 286.

4.9.2.2 The antibacterial toilet soap shall pass the test for dermatological safety when evaluated as per the method prescribed in IS 13424.

5 PACKING AND MARKING

5.1 Packing

The containers shall be supplied in suitable, well-closed containers, made of glass or plastics, or any other packaging as agreed to between the purchaser and the supplier.

5.1.1 For ECO Mark the product shall be packed in such packages which are made from recyclable/ reusable or biodegradable material and declared by the manufacturer and may be accompanied with detailed instructions for proper use.

5.2 Marking

The containers shall be legibly and indelibly marked with the following particulars:

a) Indication of the source of manufacture;

b) Volume of the material;

c) Month and year of manufacture; and

d) The following identified critical ingredients in descending order of quantity, percent by mass:

1) Total fatty matter (TFM),

2) Matter insoluble in alcohol, and

3) Antibacterial agent.

5.2.1 *Additional Marking Requirements for ECO Mark*

The package shall also be marked with the criteria for which the product has been labelled as ECO Mark.

5.2.2 *BIS Certification Marking*

The packages may also be marked with the Standard Mark.

5.2.2.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which

the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

6.1 For this purpose general precautions, scale of sampling and preparation of test samples shall be as prescribed in 3 of IS 286.

6.2 Number of Tests

6.2.1 Tests for determination of total fatty matter and free caustic alkali and matter insouble in alcohol shall be conducted on each of the individual samples separately.

6.2.2 Tests for determination of all the remaining characteristics shall be conducted on the composite sample.

6.3 Criteria for Conformity

6.3.1 For each of the characteristics which has been determined on the individual samples (**6.2.1**) the mean (X) and the range (R) of the test results shall be calculated as follows:

$$\text{Mean } (X) = \frac{\text{the sum of test results}}{\text{number of test results}}$$

Range (R) = The difference between the maximum and the minimum value of test results

The lot shall be deemed as conforming to the requirements given in **6.2.1** if the expression ($X - 0.6\,R$) is greater than or equal to minimum value given in Table 1 and ($X + 0.6\,R$) is less than or equal to maximum value given in Table 1.

6.3.2 For declaring the conformity of a lot to the requirements of other characteristics determined on the composite sample, the test results for each of the characteristics shall satisfy the relevant requirement.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A

(*Clause* 4.5.1)

DETERMINATION OF ANTIBACTERIAL ACTIVITY

A-1 GENERAL

Two methods have been prescribed, namely, serial dilution method and substantivity test. The serial dilution test shall be the screening test and the substantivity test shall be the absolute test.

A-2 SERIAL DILUTION TEST

A-2.1 Outline of the Method

Antibacterial activity is determined by serial dilution method by comparing the effectiveness of antibacterial chemicals present in 10 micrograms of soap per millilitre specified as the maximum inhibitory concentration.

A-2.2 Apparatus

A-2.2.1 *Culture Tube, Rimless*

150 mm × 18 mm.

A-2.2.2 *Sterilized Pipettes*

10 ml, 5 ml and 1 ml capacities.

A-2.2.3 *Loop Made of Stainless Steel or Platinum Wire*

A-2.2.4 *Conical Flasks*

250 ml capacity.

A-2.3 Nutrient Broth

A-2.3.1 Dissolve 5 g of beef extract, 5 g of sodium chloride, 10 g of peptone in one litre of distilled water by warming over a water bath. Cool and adjust the *p*H to 7.2 to 7.6 with sodium hydroxide solution. Distribute 9 ml each to the culture tubes. Plug the tube with non-absorbent cotton wool and sterilize in an autoclave for half an hour at 1 kg/cm^2 pressure.

A-2.3.2 Take 99 ml and 90 ml of distilled water in 250-ml conical flasks. Plug them with non-absorbent cotton wool and sterilize in an autoclave.

A-2.3.3 Get a pure stain of *Staphylococcus aureus*. ATCC 6538 P Maintain on nutrient agar medium. Transfer to a fresh slant every month and keep in the cold. Use a 24 h nutrient broth culture for the experiment.

A-2.4 Procedure

A-2.4.1 Aseptically transfer 1 g of the soap sample to the flask containing 99 ml of water. Dissolve by slight warming not exceeding 60°C. Transfer 10 ml of this solution to another flask containing 90 ml of water. Take 1 ml of this solution and add 9 ml of nutrient broth in a culture tube. This gives a concentration of 100 µg/ml.

A-2.4.2 To three tubes containing 9 ml nutrient broth add 1 ml each of the above solution to get a concentration of 10 µg of soap per ml of nutrient broth in each tube. Inoculate the tubes with a loopful of the 24 h culture of *Staphylococcus aureus* and keep them in an incubator maintained at 37 ± 2°C . Keep a control tube of nutrient broth containing the same concentration of soap.

A-2.4.3 If after 24 h incubation period, the liquid in all the three tubes is as clear as the control, the soap sample passes the test. Any turbidity more than the control shows the growth of bacteria.

A-3 SUBSTANTIVITY TEST

A-3.1 Basic Principles

For a soap to have antibacterial activity, it shall satisfy two criteria:

a) It shall show, antibacterial activity on the skin even after the soap is rinsed away, that is, the germicide should be retained on the skin under the conditions of use; and

b) The antibacterial activity should be retained on the skin for some period so as to provide protection to the skin.

A-3.2 The test devised gives a measure of both these properties. The test involves application of soap solution on the forearm, rinsing it off in running water and allowing it to dry. A mixed culture of skin flora isolated from 5 individuals (*see* **A-3.3.1**) is applied immediately in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors in 10 min is determined. Similarly the soap solution after rinsing is allowed to remain on the skin for 2 h. The test micro-organisms are applied to the skin at this time in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors is determined. If the reduction in survivors at this time is greater than 45 percent, the germicide is said to be substantive.

A-3.3 Method

A-3.3.1 *Test Micro-Organisms*

The test organisms, consist of a mixed skin flora, prepared by collecting washings from the arms and

forearms of at least 5 individuals using 50 ml of sterile water in each case. Ten ml aliquot of each washing is individually inoculated into flasks containing 90 ml of sterilized nutrient broth. Culture is allowed to grow overnight at 30°C and flask showing turbidity are pooled together. The mixed culture is transferred through broth and grown as above at least 3 times and finally maintained as Tryptone-Agar-Glucose Yeast Extract (TGYE) agar, Trypticase Soy Agar (TSA),Nutrient Agar (NA) or similar agar slants. For a test culture, an overnight slant culture is suspended into sterile saline and adjusted to a cell population of 1 × 107 cells per ml.

A-3.3.2 *Test Procedure*

A-3.3.2.1 A number of 4 cm^2 areas (2 cm × 2 cm) are marked out on the innerside of the forearm. 0.1 ml aliquot of an 8 percent soap solution with germicide is applied onto individual squares and allowed to dry for 1 min. The areas are then washed with a gentle flow of tap water for two min, dried by blowing warm air. The retentivity of the germicide on skin and its antibacterial action are then assayed by applying 0.1 ml of mixed skin flora (107 cells /ml) onto 4 such squares at 0 h. Two of the squares are swabbed immediately using standard sterile cotton swab on a stick. Swabs are placed in 5 ml saline solutions. Contents are shaken well in a vortex mixer and ten fold dilutions are prepared. Bacterial cells are assayed on TGYE agar, TSA or NA plates to determine the initial count. After 10 min, two other squares are swabbed and assayed in a similar manner.

A-3.3.2.2 In another set of tests, soap solutions are applied to the 4 more squares, rinsed and dried. After allowing 2 h interval, 0.1 ml of culture is applied as above to 4 squares. Two of the squares are swabbed and assayed at 0 h and remaining two after 10 min. Survivals at 0 h and after 2 h are determined.

A-3.4 The soap shall be considered to have passed the test if the percent kill is greater than or equal to 45 percent after two hours challenge.

ANNEX B

(*Clause* 4.5.2)

DETERMINATION OF TCC AND TCN IN SOAPS BY HPLC

B-1 PRINCIPLE

TCC and TCN are antibacterial agents, which are separated from other components in soap by normal phase or reverse phase liquid chromatography, detected spectrophotometrically and quantified by comparison with standard TCC and TCN. The method can estimate as low as 1 ppm of the above compounds.

Procedures for both normal and reverse HPLC has been described and provide the option to use either method whichever is available to the users. Both methods are comparable.

B-2 NORMAL PHASE HPLC

B-2.1 Reagents

B-2.1.1 *Iso-octane* — HPLC grade.

B-2.1.2 *Iso-propanol (2-propanol)* — HPLC grade.

B-2.1.3 *Hexane* — HPLC grade.

B-2.1.4 *Standard TCC* — 99 percent pure.

B-2.1.5 *Standard TCN* — 99 percent pure.

B-2.2 Apparatus

B-2.2.1 *High Performance Liquid Chromatograph*

Consisting of a pump, a sample injector of fixed volume with UV detector having variable wavelengths and a recorder.

B-2.2.2 *Standard Volumetric Flasks*

B-2.2.3 *Pipettes*

B-2.2.4 *Magnetic Stirrer*

B-2.2.5 *Millipore Filter Apparatus with* 0.5 *μ Filter*

B-2.2.6 *Column*

B-2.2.6.1 *Silica column*

Stainless steel 25 cm × 0.46 cm packed with Normal phase – Silica 5 μ (Lichrosorb Si-60)

B-2.2.6.2 *Cyano column*

Stainless steel 25 cm × 0.40 cm packed with (Lichrospher 100) cyano 5 μ.

NOTE — Either of the above columns can be used depending on the availability.

B-2.2.7 *Mobile Phase*

B-2.2.7.1 *For silica column*

Transfer 20 ml of *iso*-propanol into a 500-ml volumetric flask and make up to mark with *iso*-octane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B-2.2.7.2 *For cyano column*

Transfer 50 ml of HPLC grade *iso*-propanol (2-propanol) into a 500 ml volumetric flask, fill up to the mark with hexane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B-2.2.8 *HPLC Conditions*

Detector wavelength	:	280 nm
Flow rate	:	0.5 ml/min
Injection volume	:	20 µl

Retention Time

Silica Column

TCN - 7.5 minutes

TCC – 19.2 minutes

Cyano Column

TCN - 4.0 minutes

TCC – 7.5 minutes

B-2.3 Procedure

B-2.3.1 *Standard Preparation (See* Note *under* **B-3.4** *)*

Weigh accurately 25 mg of triclosan (TCN) and 25 mg of TCC into a 100-ml volumetric flask and make up to volume with the mobile phase and mix well. Pipette 1.0 ml of this solution in a 50 ml volumetric flask and dilute with mobile phase . Final concentration of TCC and TCN is 250 µg/50 ml (5.0 ppm).

B-2.3.2 *Sample Preparation*

Weigh accurately 1 g of homogenized sample into a 100-ml standard flask, and dilute to the mark with mobile phase. Pipette 10 ml of the supernatant liquid to a 50-ml volumetric flask, dilute with mobile phase, to the mark, and filter through 0.45 µm filter.

B-2.3.3 *Chromatography*

Equilibrate the column, maintained at a temperature of 30°C, with the mobile phase with a flow rate of 0.5 ml/min for *iso*-octane – *iso*-propanol mobile phase and 1.0 ml/min for Hexane – *iso*-propanol mobile phase for 30 min. Set the wavelength at 280 nm. Inject 20µl of standard solution and then sample solutions. Measure area of the peaks of respective retention time for standard and sample.

B-2.4 Calculation

$$\text{TCN, percent by mass} = \frac{\text{Area of Sample for TCN} \times \text{Conc of Standard TCN} \times 100}{\text{Area of Standard TCN} \times \text{Conc of sample}}$$

$$\text{TCC, percent by mass} = \frac{\text{Area of Sample for TCC} \times \text{Conc of Standard TCC} \times 100}{\text{Area of Standard TCC} \times \text{Conc of Sample}}$$

B-3 REVERSE PHASE

B-3.1 Reagents

B-3.1.1 *Methanol* – HPLC grade.

B-3.1.2 *Sodium Dihydrogen Phosphate Monohydrate* — Chemical grade.

B-3.1.3 *Standard TCC*

B-3.1.4 *Standard TCN (TCS)*

B-3.2 Apparatus

B-3.2.1 *Column*

Octyldimethylsilyl (C-DB)

Supercosil LC-8-DB - 15 cm × 4.6 mm. 5 µ

B-3.2.2 *Mobile Phase*

MeOH/0.01 M Phosphate buffer 62:38 *v/v*

0.01M Phosphate buffer: Dissolve 1.38 g sodium dihydrogen phosphate monohydrate in 1 000 ml of distilled water. Prepare to *p*H 3.0 by 10 percent phosphate solution.

B-3.3 Procedure

B-3.3.1 *Standard Preparation (See* Note *under* **B-3.4** *)*

B-3.3.1.1 Weigh accurately about 90 mg of TCN. Dissolve in methanol and make up to 1 000 ml volumetric flask with methanol.

B-3.3.1.2 Weigh about 110 mg of TCC, dissolve well with methanol, and make up the volume to 1 000 ml.

B-3.3.1.3 Accurately pipette 10 ml of the solution prepared in (**B-3.3.1.1**) into the (**B-3.3.1.2**) volumetric flask containing TCC. And make up to the volume with methanol. Then accurately pipette 5 ml of the solution into a 50-ml volumetric flask. Make up to the volume with methanol. Filter this standard solution through 0.45 µm filter.

B-3.3.2 *Sample Preparation*

Weigh accurately about 1.0 g of product, dissolve in methanol and make up to 100 ml in a volumetric flask with methanol. Filter this sample solution through 0.45 µm filter.

B-3.3.3 *HPLC Conditions*

Detector wavelength	:	280 nm
Column temperature	:	35° C
Flow rate	:	1.0 ml/min
Injection volume	:	10 µl

Prepare the standard solution and the sample solution at the same time. Inject the standard solution three times and calculate the average of each ingredients peak count. Inject 10 µl the sample solution and determine each ingredients percentage by the calculation shown.

B-3.4 *Calculations*

$$\text{TCN, percent by mass} = \frac{M_S \times A_r \times F}{A_S \times M_t \times 100}$$

$$\text{TCC, percent by mass} = \frac{M_S \times A_r \times F}{A_S \times M_t \times 10}$$

where

M_S = mass of the standard (g),

A_S = Averaged peak area of the standard,

M_t = mass of the test sample (g),

A_r = Peak area of the test sample, and

F = Purity of standard (percent).

NOTE — Both TCC and TCN are photosensitive, hence standards should be freshly prepared.

ANNEX C

[*Table* 1, *Sl No.*(v)]

DETERMINATION OF ZONE OF INHIBITION

C-1 OUTLINE OF THE METHOD

Antibacterial activity is determined by the zone of inhibition method by comparing the effectiveness of antibacterial chemicals present in the soap in terms of the zones of inhibition of bacterial growth with different concentrations of soap.

C-2 APPARATUS

C-2.1 Culture Tubes, Rimless

150 mm × 18 mm and 100 mm × 12 mm.

C-2.2 Sterilized Pipettes

10 ml, 5 ml and 1 ml capacities.

C-2.3 Sterilized Petri Plates

Diameter 9 cm.

C-2.4 Sterilized Filter Paper Discs

Whatman filter paper (No.1) diameter 6 mm.

C-2.5 Stainless Steel Forceps

C-3 REAGENTS

C-3.1 Dissolve 15 g of tryptone, 5 g of soya peptone, 5 g of sodium chloride in 1 litre of distilled water by warming over a water-bath. Cool it to room temperature and adjust the *p*H to 7.2 to 7.6 with sodium hydroxide. Dispense in tube/flask. Plug with non-absorbent cotton and sterilize in an autoclave for 20 min at 103 kPa pressure and 120°C. Alternatively dehydrated media may be used.

C-3.2 Tryptone Soya Agar

Prepare tryptone soya broth and add 20 g of agar powder. Alternatively dehydrated media may be used.

C-3.3 Get a pure culture of *Staphylococcus aureus* ATCC 6538 P. Maintain on nutrient agar medium. Transfer to a fresh slant every month and keep in the cold. Use an 18-24 h tyrptone soya broth grown culture for the experiment.

C-4 PROCEDURE

Inoculate a loopful of *Staphylococcus aureus* in 5 ml of tryptone soya broth or nutrient broth and incubate at 37°C for about 18 h. Towards the end of this period, a count of about 2.8 × 108 is obtained. Add 0.5 ml of the 18 h culture of *Staphylococcus aureus* to 20 ml of sterile tryptone soya agar (held at 50°C), mix thoroughly and pour into sterile petri plates. Prepare various dilutions of liquid soap in sterile saline (1 : 10, 1 : 100, 1 : 500, 1 : 1 000). Pipette 10 micro litres of these dilutions onto sterile filter paper discs, dry in air for 5 min and place onto the seeded plates. Incubate the plates at 37°C for 24-36 h. Measure the zone of inhibition around the disc.

The soap shall be considered to have passed the test if dilutions higher than 1 : 1 000 give a clear zone of growth inhibition around the disc.

ANNEX D

[*Table* 1, *Sl No.* (vi)]

BACTERIAL KILL EFFICACY ON CONTACT

D-1 PRINCIPLE

Antibacterial activity of the liquid soap is determined by measuring its rate of kill against a selected organism.

D-2 APPARATUS

D-2.1 Culture Tubes, Rimless

150 mm × 18 mm.

D-2.2 Sterlized Pipettes

10 ml, 6 ml and 1 ml capacities.

D-2.3 Loop Made of Stainless Steel or Nichrome Wire

D-2.4 Conical Flasks

100 ml capacity.

D-3 REAGENTS

D-3.1 Tryptone Soya Broth

Dissolve 15 g of tryptone, 5 g of soya peptone, 5 g of sodium chloride in 1 litre of distilled water by warming over a water-bath. Cool and adjust the *p*H to 7.2 to 7.6 with sodium hydroxide solution. Dispense 5 ml in tube. Plug with non-absorbent cotton and sterlize in an autoclave for 20 min at 103 kPa pressure and 121°C. Alternatively dehydrated media may be used.

D-3.2 Tryptone Soya Agar

Prepare tryptone soya broth and add 20 g of agar powder. Alternatively dehydrated media may be used.

D-3.3 Get a pure strain of *Staphylococcus aureus* ATCC 6538 P. Maintain on nutrient agar medium. Transfer to a fresh slant every month and keep in the cold. User an 18-24 h tryptone soya broth grown culture for the experiment.

D-4 PROCEDURE

Inoculate a loopful of *Staphylococcus aureus* ATCC 6538 P culture in tryptone soya broth. Incubate at 37°C for 18 h. Dilute liquid soap in saline to obtain 10 percent solution. To 25 ml of soap solution, add 1 ml of *Staphylococcus aureus* culture grown in tryptone soya broth for 18 h by the same procedure as given in **C-4**. Remove a loopful of the mix at regular intervals and streak onto tryptone soya agar plates. Incubate the plates at 37°C for 24-36 h. Check for growth on the streaked plates. Viable bacteria would show colony formation. The soap shall be considered to have passed the test if the growth of test organism is inhibited by contact time of one minute.

ANNEX E

(*Foreword*)

COMMITTEE COMPOSITION

Soaps and Other Surface Active Agents Sectional Committee, CHD 25

Organization	*Representative(s)*
Drugs Controller General of India, New Delhi	DR P. DASGUPTA (***Chairman***)
	SHRI B. R. WADHAWAN (*Alternate*)
Association for Consumer Action on Safety and Health (ACASH), Mumbai	SHRI YOGESH KAMDAR
	SHRI N. G. WAGLE (*Alternate*)
Central Board of Excise and Customs, Ministry of Finance, New Delhi	CHIEF CHEMIST
	DEPUTY CHIEF CHEMIST (*Alternate*)
Central Pollution Control Board, Delhi	DR S. K. GHOSH
Consumer Guidance Society of India (Regd), Mumbai	SHRI N. G. WAGLE
	SMT R. TALWANI (*Alternate*)
Consumer Education and Research Centre, Ahmedabad	DR C. J. SHISHOO
	SHRI SANTOSH YELLORE (*Alternate*)
Department of Industrial Development, Ministry of Industry, New Delhi	SHRI SHAISH KUMAR
	SHRI B. B. SHARMA (*Alternate*)
Development Commissioner, Small Scale Industries, New Delhi	SHRI J. S. REKHI
Directorate General of Supplies and Disposals (Inspection Wing), New Delhi	SHRI P. JAYAKUMARAN
	SHRI M. A. KHAN (*Alternate*)
Federation of Associations of Small Scale Soap and Detergent Manufacturers of India, Delhi	SHRI SANTOSH KUMAR
	SHRI R. C. DOSHI (*Alternate*)
Godrej Soaps Limited, Mumbai	SHRI A. RANGARAJAN
Gujarat Detergent Manufacturers Association, Ahmedabad	SHRI S. A. PATEL
	SHRI MAHENDRA VYAS (*Alternate*)
Hindustan Lever Limited, Mumbai	DR A. N. BHAT
	DR V. R. DHANUKA (*Alternate*)
Indian Soaps and Toiletries Manufacturers Association, Mumbai	SHRI V. P. MENON
Karnataka Soaps and Detergents Limited, Bangalore	DR K. B. PATIL
	SHRI M. P. NAIK (*Alternate*)
Khadi and Village Industries Commission, Mumbai	SHRI A. A. WARSI
K.S.Krishnan Associates (P) Limited, New Delhi	SHRI K. S. KRISHNAN
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